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Total Western Diet Alters Mechanical and Thermal Sensitivity and Prolongs Hypersensitivity Following Complete Freund's Adjuvant in Mice

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Abstract: Obesity and chronic pain are often comorbid and their rates are increasing. It is unknown whether increased pain is caused by greater weight or poor diet quality or both. Therefore, we utilized a Total Western Diet (TWD) to investigate the functional and physiologic consequences of nutritionally poor diet in mice. For 13 weeks on the commercially available TWD, based on the National Health and Nutrition Examination Survey, thresholds of TWD-fed mice significantly increased in both thermal and mechanical tests. Quantitative magnetic resonance imaging revealed a significant increase in fat mass with a concomitant decrease in lean mass in the TWD-fed mice. In addition, there were significant increases in levels of serum leptin and inflammatory cytokines. After chronic pain induction using complete Freund's adjuvant, hypersensitivity was more pronounced and significantly prolonged in the TWD-fed mice. Therefore, prolonged exposure to poor diet quality resulted in altered acute nociceptive sensitivity, systemic inflammation, and persistent pain after inflammatory pain induction.

***Perspective:** These results highlight the negative effects of poor diet quality with respect to recovery from hypersensitivity and susceptibility to chronic pain. A complete understanding of the impact of diet can aid in treatment and recovery dynamics in human clinical patients.*

***Key words:** Diet, pain, mice, inflammation, hypersensitivity.*

Obesity and chronic pain are increasing morbidities that have significant public health consequences.[2] Of the many clinical components of metabolic syndrome, obesity is significantly associated with chronic pain.[23] Pain is most prevalent in the load-bearing joints[46] but is also common in non-weight-bearing joints,[23] suggesting another underlying factor.[21] It is possible that excess body weight results in a persistent proinflammatory state that worsens widespread and local pain,[2] but we hypothesize that diet may be the root of the inflammatory state that may predispose individuals to chronic pain.

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Obesity is generally the result of excess energy intake and an increase in adipose tissue stores. Adipose tissue releases the adipokine leptin, which activates the innate immune system directly.[1,9] In addition, infiltrating macrophages are often recruited by adipose tissue to release proinflammatory cytokines.[41] Thus, obesity is a proinflammatory state. In many cases, obesity is the result of diets that are energy dense and nutrient poor.[29,40] These diets are often high in carbohydrates, saturated fatty acids, and omega-6 polyunsaturated fatty acids. Carbohydrates contribute to oxidative stress[12] and saturated fatty acids directly activate toll-like receptor 4 (TLR4)[20] and the inflammasome.[35] Omega-6 polyunsaturated fatty acids are precursors for prostaglandins and have a major role in the production of cytokines (ie, tumor necrosis factor α [TNF-α] and interleukin 6 [IL-6]).[17] Thus, both a gain in adipose tissue stores and a poor-quality diet may independently contribute to a chronic inflammatory state.

In general, most preclinical explorations of diet quality have focused on metabolic outcomes related to obesity. One study,[34] using a cafeteria diet, demonstrated greater

diet-induced changes in both body fat and markers of metabolic health (ie, glucose intolerance and inflammation) compared with a high-fat diet (HFD). More specifically, the cafeteria diet led to accumulation of proinflammatory mediators in the adipose tissue and increased levels of peripheral inflammatory markers.[33,34] Furthermore, the standard HFD in mice was linked with a greater than 2-fold increase in the numbers of circulating monocytes and neutrophils.[24] Once mice were returned to a low-fat diet, they had a significant loss of visceral adipose tissue, which was accompanied by decreases in both monocyte and neutrophil levels.[24] Thus, an HFD is proinflammatory as a result of immune system activation. These findings may have a direct impact on pain because administration of cytokines elicits pain in animals,[6,10,26] and we have shown that blockade of glial receptors[36,38] and glial cells themselves,[37] the primary source of cytokines in the central nervous system, reduces pain in mice. It is unknown whether a poor-quality diet and the resulting systemic inflammation have an effect on pain sensitivity and recovery from inflammatory pain induction in mice. This is a critical gap in knowledge given the increase in obesity and the possibility that diet can negatively affect chronic pain susceptibility and recovery.

To explore the relationship between diet and pain, the present study aimed to investigate the effect of diet quality on behavioral and physiologic indices of pain and inflammation in mice. To accomplish this, the Total Western Diet (TWD) was used modeled on the National Health and Nutrition Examination Survey (NHANES).[14] The TWD contains the median values of a number of micronutrients and macronutrients from NHANES, and we believe more accurately represents the diet quality of many Americans compared with the standard HFD.

Methods

Animals

Male CD1 (ICR:Crl) mice were housed in groups of 2 under a 12-hour light cycle (lights on at 07:00 AM) and provided with standard chow (Envigo [formerly Harlan Teklad], Cambridgeshire, UK) and sterile water (Hydropac, Seaford, DE) ad libitum. Male mice were used in this study based on pilot data showing that male mice showed greater diet-induced weight gain. All mice were fed standard chow for 2 weeks before introduction to an experimental diet. On assignment of diets, mice were either assigned to ad libitum AIN-93M (Harlan Teklad) chow as a maintenance and comparator diet (control, n = 8) or provided with commercially available TWD (n = 10).[14] The TWD has fewer calories from protein and carbohydrates and increased calories from saturated and monounsaturated fats than the control diet.[14] The diet exposure lasted for 13 weeks. In contrast to a typical HFD (60% kcal from fat, 20% from carbohydrates), the TWD has 34.5% and 54.5% daily kcal from fat and carbohydrates, respectively. Thus, the TWD is a high-carbohydrate diet, as opposed to an HFD. All the animals used in the present study were obtained, housed, cared for, and used in accordance with the guidelines of the University of Alabama at Birmingham Institutional Animal Care and Use Committee.

von Frey Testing

Mice were placed individually in transparent Plexiglas cubicles placed on a perforated metal floor and habituated for 2 hours before behavioral testing. Nylon monofilaments (Touch Test Sensory Evaluator Kit 2–9 [Stoelting, Wood Dale, IL]; ≈.015–1.3 g) were firmly applied to the plantar surface of the hind paw. Both paws were tested and data presented represent the average of the 2 paws. The up-down method of Chaplan and Dixon[5,8] was used to estimate 50% withdrawal thresholds. Mice were tested only when alert or resting. Testing for mechanical sensitivity was carried out at baseline and once per week during diet exposure.

Radiant Heat Paw Withdrawal Testing

Thermal sensitivity was tested using a modified Hargreaves method.[13] Mice were placed individually in transparent Plexiglas cubicles placed on an elevated glass table with a portable radiant heat source (IITC Inc, Woodland Hills, CA) under the glass table. The heat source was focused on the ventral surface during testing. Both paws were tested, and data presented represent the average of the 2 hind paws. The withdrawal latency was defined as the time to withdraw the hind paw from the heat source with a maximum of 40 seconds set as the cutoff point.

Quantitative Magnetic Resonance Imaging

At the end of 13 weeks of TWD exposure, the mice were sent to the Small Animal Phenotyping Subcore at the University of Alabama at Birmingham. Body composition (fat and lean mass) was measured in vivo using quantitative magnetic resonance (QMR) (Echo 3-in-1; Echo Medical System, Houston, TX). Three hours before the scans, food was removed from the cage. This fasting period helped avoid any potential effect of gut fill on the body composition results.

Inflammatory Chronic Pain

After testing for mechanical and thermal sensitivity (as described earlier) on 2 separate occasions separated by at least 24 hours (after 13 weeks of TWD exposure), mice were injected with complete Freund's adjuvant (CFA) (50%, in a 20 μL injection volume) into 1 hind paw. Mice were retested 24 hours later to confirm the presence of mechanical allodynia and thermal hyperalgesia, and then on days 3, 5, 8, 11, and 14 after CFA injection.

Serum Leptin

At baseline, blood was collected by submandibular bleed,[11] and serum was isolated and frozen until the final analysis. After recovery from inflammatory pain (day 15 after CFA), the mice were killed via rapid decapitation and trunk blood was collected between 09:00 and 11:00 AM. Whole-blood (clotted for 1 hour at room temperature) aliquots were centrifuged and the supernatant